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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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MORGAN LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004

EXAMINER	
MYERS, CARLA J	

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 07/05/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/091,724	ASHKAR, SAMY	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 April 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-18 and 23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18 and 23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the amendment filed April 7, 2005. Applicant's arguments have been fully considered but are not persuasive to overcome all grounds of rejection. All rejections not reiterated herein are hereby withdrawn. This action is made final.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-10, 12, 14-18 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huang (U.S. Patent No. 5,516,637; cited in the IDS) in view of Clark-Curtiss (Methods in Enzymology (1983) 101: 347-362).

Huang teaches methods for identifying host cells bound to a binding partner and methods for synthesizing, detecting and characterizing a fusion protein wherein the methods comprise: (i) expressing a fusion protein in a host cell wherein the fusion protein is encoded by a chimeric gene comprising a first DNA fragment encoding a first peptide that mediates attachment of the fusion protein to the outer membrane and a second DNA fragment encoding a second target peptide; (ii) contacting the host cell with a binding partner so as to form a complex between the fusion protein/host cell and the binding partner and detecting the host cells bound to the binding partner (see columns 5-7). In particular, Huang teaches that the first DNA fragment encodes for a

protein that includes a signal sequence that allows for the fusion protein to be expressed and displayed on the cell surface (column 6). Preferably, the first DNA fragment includes the signal sequence of the TraA gene (column 6). Huang (column 6) further teaches that the second DNA fragment encodes for a peptide capable of forming a complex with a binding partner, wherein the binding partner may be a peptide, protein, receptor, ligand or enzyme. Additionally, Huang teaches that the fusion protein/host cell/binding partner complex may be isolated and separated from unbound material by affinity separation techniques (see, e.g., column 8, liens 43-61). Huang teaches performing the method using bacteria host cells to express the fusion protein, but does not teach using bacterial minicell hosts to express the fusion protein.

However, Clark-Curtiss teaches methods for expressing heterologous proteins present in a plasmid using minicell hosts. The reference teaches methods of making minicells and teaches that E. coli, Vibrio, Erwinia and Bacillus have been used to express heterologous proteins from plasmids (e.g., page 347). Clark-Curtiss teaches that minicells are produced continually during growth of the bacterial culture and contain RNA and protein, but little or no chromosomal DNA. Consequently, while recombinant proteins synthesized in bacterial cells are often unstable due to proteolytic degradation, proteins expressed by minicells may be more stable (see, e.g., page 351 and 361). Additionally, the reference teaches that minicells can be used to assay for proteins expressed and displayed on the outer membrane of minicells (page 361).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Huang so as to have used

bacterial minicells in place of bacterial cells to express the fusion protein. The ordinary artisan would have recognized that synthesis of the fusion protein in minicells would have ensured a higher stability of the fusion protein and would have enhanced the specificity of the detection of the fusion protein because minicells are not subject to the high background of chromosomal gene products which occurs when using bacterial cells. Accordingly, one of ordinary skill in the art would have been motivated to have used minicells in the method of Huang in order to have provided a more sensitive and accurate means for detecting fusion proteins having target peptides which bind to a binding partner.

With respect to claim 5, Huang teaches that the second DNA fragment encodes for a peptide from a DNA library (see column 9). With respect to claim 12, Huang teaches that hosts harboring the selected fusion protein may be isolated, and the DNA encoding the fusion protein separated and further characterized by sequencing or affinity analysis (see, e.g., column 9, lines 20-23 and column 12). With respect to claims 14 and 15, Huang teaches that expression of the fusion protein is controlled by an inducible promoter element, such as lac (see, e.g., Figure 1, column 7 lines 66-67 to column 8 line 9; and column 8, lines 22-25).

With respect to claims 16-18, Huang states that "The fusion protein can be detached from selected cells. If desired, the target protein may be separated from the pilin protein and further purified" (see column 2, lines 46-48). Huang also teaches that the target peptide (i.e., the second peptide) may be cleaved from the fusion protein with a protease and monitored by SDS-PAGE or by examining other properties of the

peptide (see column 7, lines 22-27). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have separated the second peptide from the minicell by cleavage and to have analyzed the second peptide using techniques known in the art, such as SDS-PAGE to determine the molecular weight of the peptide, in order to have further characterized the structural properties of the isolated peptide.

RESPONSE TO ARGUMENTS:

In the response filed April 7, 2005, Applicants traversed this rejection by arguing that Clark-Curtiss does not teach that proteins expressed in minicells are more stable than proteins expressed in bacterial cells. It is stated that Clark-Curtiss teaches only that the *lon* mutation, rather than minicell technology confers enhanced protein stability. These arguments have been fully considered but are not persuasive. It is maintained that the Clark-Curtiss, when considered as a whole, does in fact teach the benefits of using minicells to express heterologous proteins. For instance, Clark-Curtiss states that "(p)rotein products produced by recombinant molecules are often unstable in *E. coli* owing to proteolytic degradation. Mutant derivatives of mini-cell producing strains that have *lon* (capR, deg) mutations are often quite useful in such instance. When the "foreign" genetic information expressed in E. coli complements the gene defect in the minicell-producing strain, we have frequently isolated spontaneous mutants for their better growth under conditions that select for maximal expression of the genetic information. Very often these mutants will possess mutations in the lon locus, which decreases the rate or extent of proteolytic degradation of "foreign" gene products" (page

351). Thereby, Clark-Curtiss does teach that minicell strains having *lon* mutations or other mutations are advantageous in that they allow for the synthesis of proteins having greater stability. It is noted that the present claims encompass the use of any type of minicell, including the minicells of Clark-Curtiss having a *lon* mutation.

Applicants assert that Clark-Curtiss teaches that minicells can be used to study protein stability, "suggesting that stability of proteins in minicells is comparable to that in whole cells." However, the Clark-Curtiss reference does not include such a statement. Rather, Clark-Curtiss teaches that "Many proteins specified by "foreign" DNA in E. coli are unstable, being subject to degradation by various proteolytic enzymes. Plasmid-containing minicells can be conveniently used to investigate the occurrence, rate, and extent of such instability" (page 361). Since the method outlined by Clark-Curtiss is one which determines the factors which effect protein stability, relative to proteins synthesized in E. coli cells, the teachings of Clark-Curtiss would not in fact suggest that protein stability is the same in minicells as in whole bacterial cells.

Applicants further state that Clark-Curtiss teaches away from the claimed invention because this reference teaches that not all protein synthesizing systems function in minicells, possibly because of the presence of nuclease or ribonuclease activity in minicells. This argument has also been fully considered but is not persuasive to overcome the present grounds of rejection. Obviousness does not require absolute predictability but only the reasonable expectation of success. See In re Merck and Company Inc., 800 F. 2d 1091, 231 USPQ 375 (Fed. Cir. 1986) and In re O'Farrell, 7 USPQ2d 1673 (Fed. Cir. 1988). The fact that some protein synthesis systems may not

be optimally effective in minicells is not considered to be a teaching away from the use of minicells, particularly in view of the additional teachings of Clark-Curtiss regarding the effective use of minicells for synthesizing heterologous proteins. Just as applicants are entitled to claims that include some inoperable embodiments, is it acceptable for the prior art to acknowledge that a methodology is not 100% predictable. Should Applicants be limited to only exemplified embodiments in view of this unpredictability, since applicant's method for synthesizing proteins would clearly be effected in the same manner by nucleases and ribonucleases? Further, this statement in the Clark-Curtiss reference is not a general teaching away from the use of minicells since Clark-Curtiss teaches that "plasmid-containing minicells have been particularly useful for studies on the replication, recombination, and repair of plasmid DNA and for studies on the synthesis of plasmid-specified gene products" (page 347). The reference also states that "use of minicell-producing strains with specified gene defects can also be advantageous in increasing the stability of macromolecules or in the study of protein processing mechanisms" (page 348). Additionally, the reference teaches that "(m)inicells from plasmid-containing minicells-producing strains can be conveniently used to examine whether plasmid-specified proteins are located in the cytoplasmic membrane, the periplasm, or the outer membrane, or whether they are excreted to the surrounding medium" (page 361). Accordingly, Clark-Curtiss teaches the effective synthesis of heterologous proteins in minicells for a wide variety of applications and for a wide variety of proteins. Lastly, Clark-Curtiss (page 347) also teaches that minicells contain protein but "little or no chromosomal DNA." Accordingly, it is maintained that the

ordinary artisan would have recognized that synthesis of the fusion protein in minicells would have enhanced the specificity of the detection of the fusion protein because minicells are not subject to the high background of chromosomal gene products which occurs when using bacterial cells.

3. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Huang in view of Clark-Curtiss, as applied to claims 1-10, 12, 14-18 and 23 above, and further in view of Shivakumar et al. (Plasmid. 1979. 2: 279-289).

The teachings of Huang and Clark-Curtiss are presented above. In particular, Clark-Curtiss teaches that minicells may be obtained from Bacillus (page 347), but does not specifically teach minicells from *Bacillus subtilis*.

However, Shivakumar (see abstract and page 286) teaches transformation of B. subtilis minicells with plasmids and use of the transformed minicells to express recombinant polypeptides. Shivakumar also teaches that *B. subtilis* minicells are free of chromosomal DNA and are useful for study gene expression of cloned DNA fragments.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have specifically used *B. subtilis* minicells to express the fusion protein in the method of Huang because, as taught by Shivakumar, *B. subtilis* minicells provide an effective host for synthesizing recombinant polypeptides and use of *B. subtilis* minicells in the method of Huang would have provided an equally effective means for synthesizing the fusion polypeptide, while avoiding the problems of proteolytic cleavage and background chromosomal encoded proteins which occur when using nucleated bacterial cells to synthesize plasmid encoded polypeptides.

RESPONSE TO ARGUMENTS:

In the response filed April 7, 2005, Applicants traversed this rejection for the same reasons set forth in paragraph 2 above. Accordingly, the response to those arguments presented in paragraph 2 above apply equally to the present invention.

4. Claims 4 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huang in view of Clark-Curtiss, as applied to claims 1-10, 12, 14-18 and 23 above, and further in view of Georgiou (U.S. Patent No. 5,348,867).

The teachings of Huang and Clark-Curtiss are presented above. In particular, Huang teaches that the first DNA fragment contains a signal sequence that directs the encoded peptide to the cell membrane, but does not teach that the DNA fragment encodes a signal sequence encoding the 17K antigen of *Rickettsia rickettsii*.

Georgiou (see, e.g., column 3) teaches methods for synthesizing fusion proteins that are expressed on a host cell's surface. Georgiou teaches chimeric genes containing a first DNA fragment encoding a signal sequence that directs a fusion protein to be expressed and anchored to the outer membrane of the host cell. The reference teaches a number of signal sequences that may be used for this purpose and specifically teaches that the signal sequence may be obtained from the 17kDa lipoprotein from *Rickettsia rickettsii* (column 3, lines 53-68 through column 4, line 3; column 5, lines 29-56).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Huang so as to have used the signal sequence from the gene encoding the 17KDa lipoprotein of

Rickettsia rickettsii in place of the signal sequence from the TraA gene because this would have provided an equally effective signal sequence for targeting and anchoring the fusion protein to the host cell outer membrane.

RESPONSE TO ARGUMENTS:

In the response filed April 7, 2005, Applicants traversed this rejection for the same reasons set forth in paragraph 2 above. Accordingly, the response to those arguments presented in paragraph 2 above apply equally to the present invention.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (571) 272-0747. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571)-272-0745.

The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at (866)-217-9197 (toll-free).

Carla Myers
June 28, 2005

Carla Myers
CARLA J. MYERS
PRIMARY EXAMINER